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Impact of Administration of Diclofenac on Cardiac Biomarkers of Adult Male Albino Rats

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ABSTRACT

Non- steroidal anti-inflammatory drugs (NSAIDs) such as Diclofenac are in common use worldwide for the treatment of conditions such as pain, rheumatoid arthritis, and musculoskeletal disorders. The efficacy of this drug in alleviating pain has made it prime choice in the management of cancer and sickle cell disease patients. antipyresis and analgesia. In spite of their beneficial effects, NSAIDs have been notified to enhance the risk of heart attack and stroke the present study was conducted to investigate the impact of short-term administration of different doses of diclofenac on cardiac biomarkers. Twenty male albino rats weighing 150-200g were divided equally into four groups. The rats in the control group were orally administered distilled water. The other three groups were given diclofenac doses. The rats in the first (n=5), second (n=5), and third (n=5) groups were orally administered diclofenac dose of 150, 100 and 50mg/kg live weight/day, respectively, every day for seven days. At the end of the experimental period, the animals were sacrificed, blood samples were taken from the animals by Cardiac puncture under general anesthesia and afterwards rats were immediately euthanized. Cardiac tissue samples were prepared for histological assessment. Rat – specific cardiac Troponin (T) level showed that the group administered 150 and 100mg/kg Diclofenac (DIC) increased significantly ($p<0.05$) when compared to the control while that of 50mg/kg had no significant difference. Creatinine Kinase-MB, lactate dehydrogenase (LDH) and aspartate amino transferase (AST) Activity increased significantly when compared to control. This increase is dose-dependent with the highest dose having more serum activity than the low dose. The histological examination of the heart shows that the high dose imposed cardiac injury while the low dose had no effect on the heart. In conclusion, these results suggest that high dose of diclofenac may cause cardiac injury while low dose may cause little or no cardiac injury even at short term administration.

Keywords: Non- steroidal anti-inflammatory drugs (NSAIDs), Diclofenac, Cardiac biomarkers, Histology.

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1. INTRODUCTION

Drugs are chemical substances that form the foundation of therapy in human diseases. They are usually given for prevention, control or cure of diseases. Most drugs act by interacting with a cellular component called receptor and the efficacy of a drug is measured by the degree of effect it is able to generate at a receptor site (Okwakpam et al. 2018b). Drugs that exert useful therapeutic effect may also exert unwanted or toxic effects to different body organs (Okwakpam et al. 2018b).

Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) are used worldwide to treat pain, fever and inflammation. They include the nonselective or traditional NSAIDs, as well as the selective cyclooxygenase-2-specific ones (Klaassen et al. 2001). These agents are most often used to manage pain associated with musculoskeletal conditions.

Diclofenac (2,6 – Dichloroanilino Phenylacetic acid), one of the most frequently prescribed nonselective NSAIDs worldwide, has strong analgesic, antipyretic, and anti-inflammatory effects and also has the most inhibitory effect on the prostaglandin production other than NSAID (Erdal and Sefa, 2017). The efficacy of this drug in alleviating pain has made it prime choice in the management of cancer and sickle cell disease patient.

Diclofenac exerts its action by working against the enzyme cyclooxygenase (cox). Cyclooxygenase Enzymes (Cox) is mainly expressed in two forms as Cox1 and Cox2. Selective effect of diclofenac on the Cox2/Cox1 is 4/1 (Gulsen et al. 2013; Jüni et al. 2004).

The risk of side effects affecting the gastrointestinal tract and kidneys has long been known, the possibility of blood pressure elevation, increased incidence of acute myocardial infarction, and heart failure are also been recognized (Varga et al., 2017). Despite this, the nonselective or traditional agents such as diclofenac are still widely used, and also freely available as over-the-counter analgesics (Farkouh et al. 2009).

McGettigan & Henry (2013) have also reported that diclofenac has the highest cardiovascular risk score of the nonselective NSAIDs due to its he increased selectivity for COX-2. There is also increasing evidence that long-term treatment with diclofenac is associated with onset or aggravation of congestive heart failure, which can cause serious cardiovascular thromboembolic events, such as myocardial infarction and stroke (Cheetham et al. 2008; Jevdjevic et al., 2014). This conclusion might be because only diclofenac inhibits L-type Ca2+ channels and the Na+ current in cardiomyocytes (Paoloni et al. 2009).

Therefore, a considerable concern should be taken to study the toxicity of diclofenac due to its clinical use and adverse effects.

This present work is aimed at investigating the impact of short-term administration of different doses of diclofenac on cardiac biomarkers in albino rats.

2. MATERIALS AND METHODS

Reagents and Chemicals Diclofenac sodium (Diclomax-100) was purchased from TSK global pharmacy in Alakahia Port Harcourt, Nigeria by Laborate Pharmaceuticals Ltd, India All other reagents and chemicals used for the study were of analytical grade.

Experimental Animals

Twenty male albino rats weighing between 150-200g obtained from the animal house of the Department of Biochemistry, University of Port Harcourt were used to investigate the impact short term administration of diclofenac cardiac biomarkers such as specific cardiac Troponin (T), Creatinine Kinase-MB, lactate dehydrogenase (LDH) and aspartate amino transferase (AST) Activity. The animals were allowed to acclimatize for one week prior to commencement of the study. The rats were kept in well ventilated cages and fed with commercial growers' mash, manufactured by Top Feeds Ltd, Sapele, and Delta State, Nigeria. Water and feed were administered ad libitum.

Experimental Design

After the period of acclimatization, animals were randomly divided into four different groups comprising five animals in each group (n=5). The first was the control that was not administered with the drug and the others were the test groups administered with varying doses of the drug, diclofenac (DIC) according to the body weight for a period of seven days. At the end of the drug administration, animals were sacrificed by decapitation under pentobarbitone anesthesia and blood samples and heart were collected into clean specimen bottles for analysis. The heart tissues were preserved in a sample bottle containing 10% formal-saline solution for histopathological examination. Administration protocol of the drug for the animals is presented in Table 1.

Drug Administration

Different doses of diclofenac were freshly prepared in distilled water and administered to the animals orally according to the body weight.

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Table-1: Animal grouping and drug administration

Groups	No. of animals	Dose of drug administered
(Control)	5	Distilled water
Group A	5	150mg/kg/DIC
Group B	5	100mg/kg/DIC
Group C	5	50mg/kg/DIC

Determination of Biochemical Parameters

The serum level of cardiac troponin (T) was established using ELISA kit according to the procedure suggested by Farkouth and Greenberg (2009). The serum activity of creatinine kinase (CK-MB) was determined using Agappe test kits according to the method by Witt and Trendelendurg (1982). The serum activity of lactate dehydrogenase (LDH), aspartate amino transferase (AST) was determined using Agappe test kits according to the method by Thefeld, (Anon, 1972).

Histopathological Examination of the Heart

Pieces of the heart tissue were cut off and fixed in 10% Formal-saline solution. The thickness of tissues was reduced by cutting the tissue into sections, two to three cells thick of 5 – 20 microns. After which they are stained to render the various tissue and cell constituents prominent under microscopic observation (Banerjee & Bhattacharya, 1994).

3. RESULTS

Results of Troponin (T) concentration of each group is given in Table 2. The groups administered 100mg/kg and 150mg/kg (groups A and B) showed significantly increased, while the groups administered 50mg/kg (group C) had no significant difference when compared to the control. Results of Lactate dehydrogenase showed significantly increase in group C compared to control, groups A and B. Also, groups A and B showed significant ($p<0.05$) difference compared to control. Results of Creatinine Kinase activity showed significant increase in the group C compared to control and groups A and B. However, there was no significant difference in groups B and C.

Table 2: Results for troponin (T) Concentration in the Experimental Rats

Groups	Treatment	Troponin (T) (ng/ml)	Lactate dehydrogenase(U/L)	Aspartate amino transferase (U/L)	Creatinine kinase (U/L)
Control	Control	0.47 ± 0.02^{a}	9.00 ± 0.58^{a}	21.67 ± 0.88^{a}	9.00 ± 0.58^{a}
A	150mg/kg	1.09 ± 0.08^{b}	27.00 ± 1.73^{c}	45.00 ± 0.57^{b}	27.00 ± 1.73^{c}
B	100mg/kg	0.85 ± 0.19^{b}	24.67 ± 2.60^{b}	43.33 ± 2.03^{b}	24.67 ± 2.60^{b}
C	50mg/kg	0.56 ± 0.05^{a}	18.67 ± 1.20^{b}	48.33 ± 2.06^{b}	18.67 ± 1.20^{b}

Values are expressed as mean ± SEM, (n=5). Superscript having the same alphabet is not significant.

4. DISCUSSION

Diclofenac (DIC) is the most prescribed NSAIDs in the human and veterinary medicine especially because of its strong anti-inflammatory and analgestic property (Paoloni et al. 2009). In this study, the serum concentrations of troponin (T), Creatine Kinase (CK-MB), Lactate dehydrogenase (LDH) and Aspartate aminotransferase (AST) as indices of myocardium injury was investigated.

Troponin T (Tn-T) is considered to be primary marker of drugs induced cardiac cell misfortune in humans and animals (Ahmad et al. 2018). Enhanced troponin levels are important bacon for the increased coronary disease, suboptimal coronary flow and assessment of infarct size in myocardial infarction (Wells & Sleeper, 2008). It has been reported that loss of membrane integrity due to heart cell injury could be the main reason for the cardiac troponin release in the blood stream (Franceschi et al. 2016). The result from this study showed that serum level of troponin (T) increased significantly ($p<0.05$) in groups A and B that received 150mg/kg DIC and 100 mg/kg DIC respectively when compared with the control while there was no significant ($p>0.05$) difference in group C that received low dose of 50mg/kg DIC when compared to the control. This result agrees with the work conducted by Franceschi et al. (2016). Serum activity of Tn-T in rats administered with low dose of diclofenac, showed no significant increase. It has been reported that serum CK-MB, LDH and AST are well known diagnostic enzymes marker of myocardial injury (Okwakpam et al.

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2018b; Cheetham et al. 2008). Myocardial cells destruction due to insufficient oxygen supply or glucose, break cardiac membrane or make it permeable which leads to enzymes escape and enters in to blood stream (Okwakpam et al., 2018a).

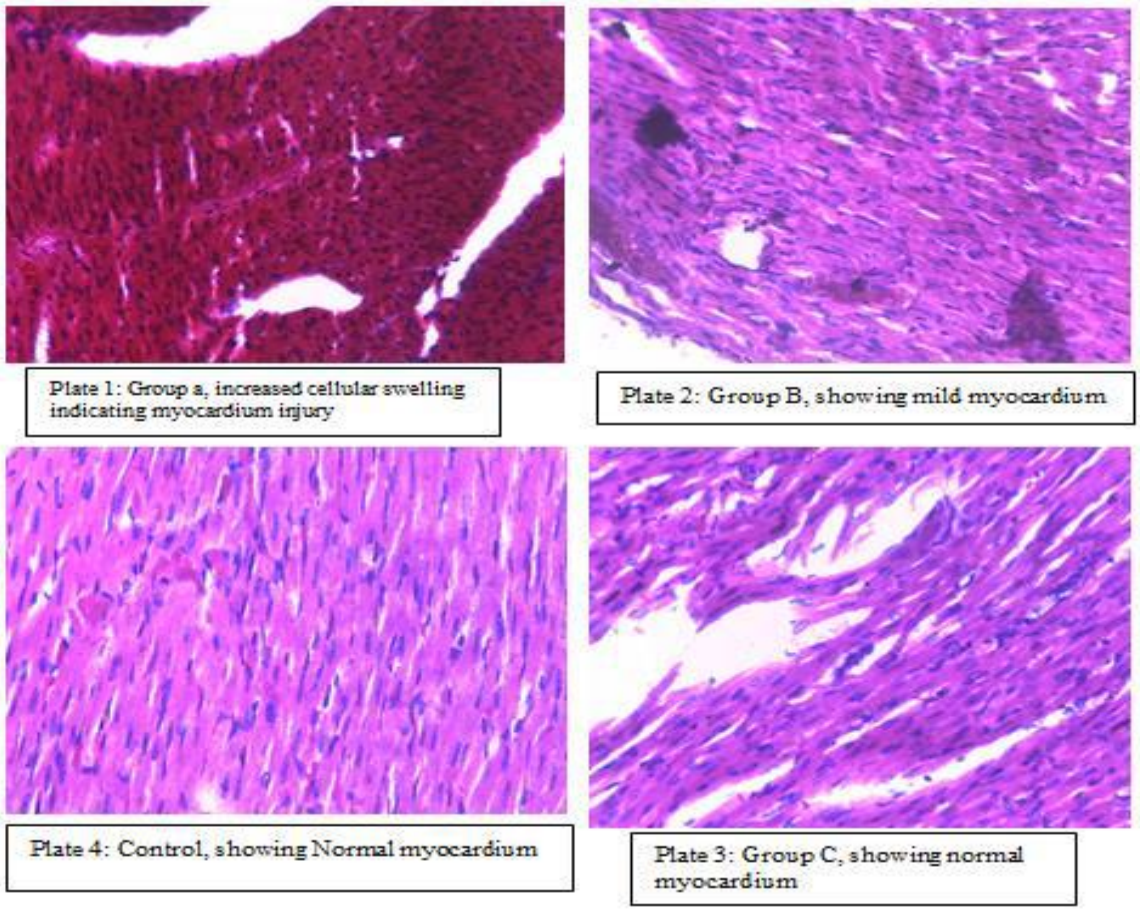

Fig-1: Histological features of the heart of the experimental animals

Creatine Kinase (CK-MB) is an intracellular enzyme that is involved in cellular energy transport (Clark, 2013). Increased serum activity of CK-MB defines myocardial necrosis (Oudman et al 2013). Results showed that the administration of diclofenac significantly ($p<0.05$) elevated serum level of CK-MB in all the test groups when compared to control, which is an indication of cardiac injury. This result agrees with the work of Erdal and Sefa, (2017). Diclofenac increased the serum activity of CK-MB in rats in a dose dependent manner. Lactate dehydrogenase (LDH) an intracellular enzyme predominant in the cells of the liver, brain, cardiac and skeletal muscles, kidney and erythrocytes. It is used as secondary marker in determining heart damage and therefore an essential tool in clinical analysis to detect cardiac injury (Jose & Maden, 2013). Result obtained shows that administration of diclofencac significantly ($p<0.05$) increased serum activity of LDH in the test group when compared to control. A high level of LDH in the serum is an indicator of myocardial injury. Aspartate aminotransferase is an intracellular enzyme present in the liver, brain, kidney, heart and muscle. It is used as a secondary marker in determining heart damage (Jose & Maden, 2013). Results obtained from this study indicated significant ($p<0.05$) increase in serum activity of AST in all test groups when compared to control group and this may lead to cardiac injury. Although AST is also available in other tissue such as brain, kidney and muscle, elevated AST are considered as important markers of cardiac injury (Erdal and Sefa, 2017). The histological observation of cardiac tissue of the control and the test group administered 50mg/kg DIC showed no damage to the heart, which implies a normal myocardium. The test groups administered 100mg/kg DIC and 150mg.kg body weight showed mild and severe cellular swelling respectively which is an indicative of myocardial injury. The results obtained from the study are similar to studies conducted by work of Gulsen et al. (2013). Administration of high dose Diclofenac caused cardiac injury, while the administration of low dose Diclofenac has no effect on the heart. Thus, it can be deduced that that oral administration of Diclofenac may lead to cardiac injury, most especially when high doses are administered in humans.

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Conflict of Interest:

The authors declare that there are no conflicts of interests.

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This study has not received any external funding.

Ethical approval

The Animal ethical guidelines are followed in the study for experimentation.

Data and materials availability:

All data associated with this study are present in the paper.

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